(54) Title: METHODS FOR TREATMENT OF DISEASES ASSOCIATED WITH INFLAMMATION UNDER NON-ISCHEMIC CONDITIONS

(57) Abstract: The present invention relates to a method for treatment or prevention of a non-ischemic condition in one or more organs, the method comprising administering an effective dosage of α-MSH and/or an α-MSH equivalent and/or EPO and/or an EPO equivalent to a person in need thereof. In particular, the invention relates to the treatment of inflammation under non-ischemic conditions. Non-limiting examples of such conditions are: Asthma, arthritis, psoriasis, infections, systemic lupus erythematosus, Systemic Sclerosis, allergic rhinitis, allergic and non-allergic conjunctivitis. Moreover, inflammatory diseases also include allergic and non-allergic dermatitis.

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Methods for treatment of diseases associated with inflammation under non-ischemic conditions

FIELD OF INVENTION

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The present invention relates to a method for treatment or prevention of inflammation in a non-ischemic condition in one or more organs, the method comprising administering an effective dosage of α-MSH and/or an α-MSH equivalent and/or EPO and/or an EPO equivalent to a person in need thereof. In particular, the invention relates to the treatment

10   of inflammation under non-ischemic conditions.

The present invention relates to such treatment with respect to all organs in the human or animal body, and in particular to treatment of airways, lung, kidney and urinary tract. In this respect, 1) single compound treatment (either α-MSH or EPO) of e.g. asthma,

15   ureteral obstruction and nephritic syndrome being non-ischemic conditions, and 2) combination treatment with α-MSH equivalents and epoetin-alpha of asthma, ureteral obstruction and nephritic syndrome are contemplated. However, it appears that treatment with a combination of α-MSH and/or an α-MSH equivalent and EPO and/or an EPO equivalent has a marked effect which is significantly better than single compound

20   treatments. Accordingly the invention also relates to a pharmaceutical composition including a kit comprising the combination.

BACKGROUND

25   *Melanocortins*

Melanocortins are proopiomelanocortin-derived mammalian peptide hormones that include adrenocorticotropic hormone [ACTH (1-39)], α- melanocyte-stimulating hormone [α-MSH (1-13)], and related amino acid sequences including β- and γ-MSH. Melanocortin peptides have potent antiinflammatory/anticytokine activity (Lipton and Catania

30   *Immunol.Today*, 18: 140-145, 1997). Melanocortins excert at least some of their effect via stimulation of melanocortin receptors. For melanocyte stimulating hormones (MSH) the action is in part ascribed to binding and activation of type 1-5 melanocortin receptors (MC1-MC5).

Melanocortins have a variety of functions including immunomodulation, anti-inflammation, body temperature regulation, pain perception, aldosterone synthesis, blood pressure regulation, heart rate, vascular tone, brain blood flow, nerve growth, placental development, synthesis/release of a variety of hormones such as aldosterone, thyroxin,

5 prolactin, FSH. ACTH has a major effect on stimulating steroidoneogenesis. Also α-MSH induces pigment formation in skin.

Five genes encoding melanocortin receptor subtypes have been identified (MC-receptor type 1-5). The MC receptors belong to the class of G-protein coupled receptors and have

10 seven membrane spanning domains. All receptor subtypes involve increased production of camp to excert their actions. Type 2 receptor (MC2) represent the ACTH receptor whereas the other subtypes are melanocyto stimulating hormone receptors (MSH-receptors).

15 A series of studies have been performed on the MC receptors in a variety of tissues. Type 1 receptor (MC1), to which α-MSH binds with great affinity, is known to be expressed in several tissues and cells such a brain, including astrocytes, testis, ovary, macrophages, neutrophils. However MC1 is likely to be expressed in an even wider range of tissues although this remains to be established. The selectivity for the MC=s to bind different

20 melanocortin peptides vary. MC1 binds with great affinity α-MSH and with lower affinity also β-MSH, γ-MSH and ACTH. MC2 has been reported only to bind ACTH but non of the MSH peptides. The highest affinity for the ligands of the other receptors including γ-MSH (MC3-receptor), β-MSH (MC4-receptor). In contrast MC5 binds with much lower affinity the MSH peptides but with the same pattern as MC1 (i.e. highest affinity for α-MSH).

25

It is important to emphasize that a number of actions of MSH peptides, especially α-MSH, are not fully established with respect to which receptors are involved. The anti-inflammatory action of α-MSH has been speculated to involve a variety of processes including interference with NO production, endothelin-1 action, interleucin 10 formation,

30 which again is linked to MC1 receptors expressed in macrophages, monocytes.

α-MSH has also been shown to be important in a variety of infllamtory processes (Lipton and Catania 1997): 1) Inhibit chemotactive migration of neutrophils (Catania1996). 2) α-MSH including analogs inhibit the release of cytokine (IL-1, TNF-α) in response to LPS

treatment (Goninard1996). 3) Inhibit TNF-α in response to bacterial endotoxin (Wong, K.Y. et al. *Neuroimmunomodulation*, 4: 37-41,1997). 4) ICV or IP administration of α-MSH inhibit central TNF-α production by locally administered LPS. 5) α-MSH has been shown to reduce the inflammation in experimental inflammatory bowel disease (Rajora, N. et al.

5   *Peptides*, 18: 381-385, 1997), ishemia-induced acute renal failure(Star, R.A. et al. *Proc.Natl.Acad.Sci.U.S.A*, 92: 8016-8020, 1995). 6) α-MSH also have some protective effect by inhibiting the induction and elicitation of contact hypersensitivity and induces hapten tolerance, and it is speculated that α-MSH may mediate important negative regulation of cutaneous inflammation and hyper-proliferative skin diseases (Luger, T.A.

10 *J.Investig.Dermatol.Symp.Proc.*, 2: 87-93, 1997. To this end α-MSH causes increased IL-8 release from dermal microvasculature endothelial cells (Hartmeyer, M. *J.Immunol.*, 159: 1930-1937, 1997).

Erythropoetin (EPO)

15 The cellular adaptation to hypoxia involves many changes in gene expression, such as those of erythropoietin (Epo), vascular endothelial growth factor (VEGF), glycolytic enzymes, and tyrosine hydroxylase. Several reports have demonstrated that both oxygen sensing and chemical signaling occur via a common pathway that leads to the activation of hypoxia-inducible factor-1 (HIF-1), a transcription factor which is induced over a

20 physiologically relevant range of oxygen tensions Epo is a 34-kDa glycoprotein hormone which has been characterized as the principal regulator of erythropoiesis and was thought to be exclusively produced in fetal liver and adult kidney in response to hypoxia . The molecular biology of the oxygen sensing mechanism underlying the transcriptional activity of Epo has been intensively investigated in HepG2 and Hep3B human hepatoma

25 cell lines. In addition to transcriptional activation by HIF-1, mRNA stabilization has been found to account for an accumulation of Epo mRNA. Agents such as cobalt chloride (CoCl2) and desferrioxamine (DFX) are able to mimic the hypoxia-induced Epo transcription.

30 Indirect evidence has been provided to indicate that redox-mediated processes are likely to be involved in the induction of the EPO gene. Thus, iron and reactive oxygen species might play a critical role in the oxygen sensing mechanisms involved in the regulation of the expression of the EPO gene. Recent reports suggest that, along with its role in erythropoiesis, EPO might be of biological significance in the central nervous system. In

35 vivo, EPO mRNA is expressed in both rodent and primate brain tissues and its expression

is increased following hypoxia. Taken together, several findings imply that EPO acts on neurons in a paracrine way. This notion has been supported by the in vitro and in vivo europrotective effects of Epo. Several groups (Sadamoto, Y. et al. *Biochem. Biophys. Res. Commun.*, 253: 26-32, 1998, Sakanaka, M. et al. *Proc.Natl.Acad.Sci.U.S.A*, 95: 4635-4640,

5 1998, Bernaudin, M. et al. *J.Cereb.Blood Flow Metab*, 19: 643-651, 1999) have shown that the direct administration of EPO to the central nervous system of mice, rats, and gerbils to some extent reduces neuronal death and prevents learning disability associated with cerebral ischemia.

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BRIEF DESCRIPTION OF THE INVENTION

The effect of treatment with i.v. α-MSH equivalents alone, epoetin alone or α-MSH equivalents and epoetin combined was established in models of lung inflammation,
15 systemic inflammation, kidney inflammation, and diseases of the urinary tract including the bladder. Models include LPS administration, ureteral obstruction, and aminoglycoside induced nephrotic syndrome. Various functional parameters were determined and the expression levels of relevant transporters were monitored to establish the effect of these compounds in these settings.

20

Acute/subacute LPS-inhalation induced lung and airway inflammation.
Treatment with the compounds significantly prevented the neutrophil and eosinophil airway and lung infiltration. Subacute systemic administration of LPS gave multiple organ inflammation including renal inflammation and treatment with the compounds significantly
25 prevented the development of severe renal failure. Functional parameters of kidney function and of renal transporter expression were determined as parameters of treatment efficiency. Temporal ureteral obstruction for 24 hours (uni or bilateral) followed by release of obstruction for various time periods (1-30 days) induced significant changes in kidney and urinary tract function. Markers include functional parameters, downregulation of renal
30 water and sodium transporters. Treatment with i.v. α-MSH -equivalents alone, epoetin alone or α-MSH and epoetin combined markedly reduced the downregulation of the renal marker proteins and prevented the reduction in kidney and urinary tract function.

In rat models of purimycin or adriamycin induced nephrotic syndrome ascites and
35 proteinuria developed as prominent signs of severe nephritic syndrome. Other markers

included downregulation of renal water channels (aquaporins) and sodium transporters. Treatment with i.v. α-MSH-equivalents alone, epoetin alone or α-MSH and epoetin combined markedly reduced the downregulation of the renal marker proteins and reduced proteinuria and ascites production.

5

Inflammation of airways and lung is often associated with swelling of airway and lung tissue and infiltration of airway and lung tissue with leucocytes including neutrophils, eosinophils or basophils and mast cells. This is well established and seen in extrodinary common conditions such as common cold, airway infection, and pulmonary infections, but

10 also in conditions associated with allergy including allergic rhinitis, asthma or other allergic conditions. Moreover this is also seen in acute diseases associated with inflammation of lung and airways as well as in chronic obstructive pulmonary disease (COPD). At least three conditions contribute to COPD. (1) Chronic bronchitis is an inflammatory condition in which neutrophils, CD8+ T-lymphocytes and CD68+ monocytes/macrophages

15 predominate. The condition is defined clinically by the presence of chronic cough and recurrent increase in bronchial secretion sufficient to to cause expectoration. There is enlargement of mucus-secreting glands and goble cell hyperplasia, which can occur in the absence of airflow limitation. (2) Adult chronic bronchiolitis is an inflammatory condition of small bronchi and bronchioli in which here are CD8+ and macrophages. (3) Emphysema

20 is an inflammatory condition of the alveoli in which neutrophils, T-lymphocytes, and macrophages/monocytes are involved, associated with the release of excessive amounts of elastases from neutrophils.The course of COPD is characterized by intermittent exacerbations of the disease. In an axcerbation, there is also an significant influx of eosinophils into the tissue contributing to the inflammation. COPD is the fourth leading

25 cause of death in the United States. The incidence, morbidity, and mortality of COPD is rising throught the world. The total economic cost of COPD in the US in 1993 was estimated to be over $US15.5 billion. Treatments effectively reducing or stopping the progress of COPD are needed. The ideal therapy in COPD would be compounds capable of reducing the influx of neutrophils into the airways or shrinking the enlarged mucous

30 producing glands. As the inflammation continues natural repair processes start, resulting in peribronchial fibrosis, loss of lung elasticity and emphysema.
Also inflammation seen in other organs (either local) or attributed to systemic or more widespread inflammation is a feature associated with many diseases including infections, allergy, rheumatic diseases, cancer and other conditions and diseases or as a side-effect

35 of drugs or poisoning.

In some cases of systemic inflammation this often involves inflammation or affection of the kidney leading to renal dysfunction or renal failure often seen in severe life-threatening conditions. The reduction in creatinine clearance or other renal functional parameters are

5   indicative of such systemic inflammation.

Bilateral ureteral obstruction (BUO) is associated with reduction in renal functions. Characteristically, long-term loss of urinary concentration capacity is a common finding in both children and adults. The present inventors and others have shown that BUO and

10   release of BUO in rats is associated with the onset of a dramatic postobstructive diuresis (POD) and a reduction in urinary concentrating capacity. Importantly, it was demonstrated that a BUO and release of BUO in rats was associated with a marked reduction in the protein expression of aquaporin-2 (AQP2), AQP3 and AQP1. Unilateral ureteral obstruction (UUO) is a model of renal injury characterized by progressive tubulointerstitial

15   fibrosis and renal damage, while relatively sparing the glomerulus and not producing hypertension or abnormalities in lipid metabolism. Irrespective of the underlying cause, many kidney diseases lead to tubulointerstitial inflammation and eventual interstitial fibrosis with permanent loss of renal function. Most medical investigators agree that new therapeutic strategies should be targeted at developing effective methods for inhibiting

20   renal fibrogenesis. The mechanisms responsible for UUO-induced kidney fibrosis are not well understood. However, prolonged obstruction induces progressive renal fibrosis with dysfunction, which cannot be readily restored even with removal of the obstruction. Under these circumstances, pharmacotherapeutic intervention needs to be developed to reverse or halt the progression of the renal dysfunction that occurs as a consequence of the

25   obstruction. Furthermore, the development of progressive interstitial fibrosis represents a final common pathway associated with a variety of kidney disorders that can lead to functional insufficiency. Thus, the search for effective treatment preventing the progression is of great importance not only for elucidating the mechanism of UUO-induced fibrosis, but also for alleviating the renal fibrosis seen under various conditions

30   with chronic renal failure (CRF).

UUO results in changes in renal hemodynamics, infiltration of the kidney by macrophages, and subsequent fibrosis of the tubulointerstitium. Many of the pathophysiological alterations associated with renal disease are driven by the intercrine, autocrine, paracrine, and endocrine effects of angiotensin II and it has been have demonstrated that

35   angiotensin II production is rapidly stimulated following the onset of ureteral obstruction.

Angiotensin II, in turn, upregulates the expression of other factors including transforming growth factor- (TGF-), tumor necrosis factor- (TNF-α), nuclear factor-B (NF-B), adhesion molecules, and chemoattractants , matrix proteins , and -smooth muscle actin (-SMA). The role of TNF- in the pathophysiology of obstructive uropathy, when compared with

5 angiotensin II, is not well understood. In rats, pharmacological manoeuvers has been applied to inhibit angiotensin II formation or its biological action through receptor inhibition. No such pharmacological treatments are available to decipher the biological actions of TNF-. Two different cell surface receptors exist for TNF-, which are designated TNFR1 and TNFR2, that are derived from separate gene products. Moreover it has been shown

10 that TNF- contributes, in part, to changes in interstitial volume, myofibroblast differentiation, and NF-B activation in the kidney during ureteral obstruction and are mediated through both the TNFR1 and TNFR2 gene products (mouse study). Thus the angiotensin II and TNF- systems appear to interact with each system, contributing to overall renal fibrosis. Also apoptosis plays a role and it has been shown that the

15 expression of apoptotic and chemokine genes are significantly upregulated in UUO, and bioflavonoids and angiotensin inhibitors are able to attenuate the expression of these genes and thus may be beneficial in renal protection.

There are no really good treatment of these conditions: 1) The effect of anti-inflammatory

20 treatment with corticosteroids (methylprednisolone) in patient with urinary tract obstruction caused by stones showed that treatment alone did not affect stone passage but combined treatment with the calcium antagonist treatment (nifedipine + methyl prednisolone) facilitated ureteral stone passage. In a few other studies it has been shown that corticosteroid treatment most likely is able to reduce the oedema of the ureteral wall

25 associated with ureteral obstruction. 2) The effect of nonsteroidal anti-inflammatory treatment with cyclooxygenase inhibitors such as indomethacin, toradol and sulindic acid, agents which block prostaglandin synthesis, all have been shown to have some beneficial effect on stone passage and reduce pains associated with ureteral obstruction. The mechanism involved are speculated to be due to a direct effect on ureteral

30 contraction, reduced oedema of the ureteral wall which in turn may reduce ureteral pressure. The effect to reduce pain by effecting ureteral pressure may also be due to a direct side effect on renal function where GFR and RBF are reduced due to blockade of the effects of vasodilating prostaglandins on renal hemodynamics. Thus a drug with major effect on renal and urinary tract function during obstruction and other kidney disorders

35 associated with fibrosis etc is warranted.

Another common cause of renal failure is nephrotic syndrome, which is caused by glomerular damage and can be a result of treatment with drugs such as adriamycin or purimycin (PAN) aminoglycosides (and other drugs) but the underlying cause of most

5 cases remains unidentified and many patients progress into renal failure (for references see). Nephrotic syndrome is associated with severe proteinuria, systemic edema including ascites, hypoproteinemia and hyperlipidemia. It is also associated with decreased urinary concentrating capacity and dilutional ability, and severe sodium and water retention is a cardinal feature in nephrotic syndrome leading to ascites, and progression into renal

10 failure. The intrarenal factors leading to the dysregulation of kidney function are not well understood and currently there is no good treatment that prevents the progression of kidney damage. The glomerular changes and possibly also the tubular changes that results in glomerular and tubular dysfunction leading to heavy proteinuria, sodium and water retention and massive peripheral edema have been speculated to be secondary to

15 infiltration with neutrophils, macrophages and monocytes (and induction of many cytokines and adhesion molecules), although this remains less well defined. Also insufficient proliferation and apoptosis in glomerular epithelial cells may be involved in the progression. Thus an inflammatory response is likely to contribute during some stages of the development of renal failure.

20

A number of renal transporters and channels have been shown to be dysregulated in association with nephrotic syndrome and this is likely to contribute to the derangement in kidney function. It has been speculated that the decrease in transporter expression in experimental nephritic syndrome is a direct effect of the causing agents (e.g. adriamycin

25 or PAN) on the renal tuble and glomerual epithelial cells. The mechanism by which this agent produces obliteration of the foot processes of glomerular podocytes is not known, but the effect presumably involves impairment of the vesicle trafficking processes involved in maintaining the complex shape of these cells. It appears possible that the decline in ion transporter and water channel expression induced by adriamycin is a consequence of a

30 similar impairment of trafficking in renal tubule cells although this is not established.

Nephrotic syndrome differs markedly from ishemia-induced acute renal failure, which is due to reduced or complete arrest in blood supply to the kidney(s). Nephrotic syndrome is often caused (as described above) by drugs (aminoglycosides and other antibiotics),

35 infections, autoimmune disease, connective tissue diseases, cancer and many immune-

mediated forms of glomerulonephritis and is never induced by ischemia. Ischemia is mainly associated with tubular damage whereas nephritic syndrome is mainly a glomerular disease with secondary affection of the tubular system. Thus the etiology is completely different.

5

As decribed above (and in the Lipton review) α-MSH is likely to exert its effect via MC-1 receptors expressed in inflammatory cells (including neutrophils) or directly in the epithelium (not known). The effects of epoitin outside its effect in stimulating erythropoesis is virtually undefined but may include leads activation of hypoxia-induc-ible factor-1 (HIF-

10   1), a transcription factor which is induced over a physiologically relevant range of oxygen tensions. EPO receptors have been found in multiple tissues including kidney and it is speculated that EPO acts via this receptor although binding to other receptors cannot be excluded.

15   DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for treatment or prevention of an inflammatory condition in one or more organ(s) or tissue(s) such as treatment or prevention of the various conditions above. The organs include, but are not limited to, the airways and lung,

20   the kidney and urinary tract system and the prostate. The method comprises administration of an effective dosage of α-MSH and/or of an α-MSH equivalent to the individual in need thereof. In another embodiment, the invention relates to the administration of an EPO or EPO equivalent to the individual in need thereof. In a still further and preferred embodiment, the invention relates to the use of both EPO and/or

25   EPO equivalent and α-MSH and/or an α-MSH equivalent for the treatment or prevention of an inflammatory condition in one or more organ(s) or tissue(s) wherein a combination is administered to the individual in need thereof. In the present context, the term medicament may accordingly represent either α-MSH and/or α-MSH equivalent, EPO and/or EPO equivalent as well as any combination thereof. An example of a such

30   combination is α-MSH together with an EPO equivalent.

By the term "an inflammatory condition" is in the present context meant a condition in which mechanisms such as reaction of specific T lymphocytes or antibody with antigen causes the recruitment of inflammatory cells and endogenous mediator chemicals. In

35   some cases, the normal function of the organ or tissue will be altered by an increase in

vascular permeability and/or by contraction of visceral smooth muscle. Such inflammatory conditions may give rise to inflammatory diseases.

Inflammatory diseases include the following diseases (non-limiting list): Arthritis, including
5   diseases associated with arthritis), osteoartritis, rheumatoid arthritis; spondylarthropathies (e.g. ankylosing spondilitis), reactive arthritis (including arthritis following rheumatic fever), Henoch-Schonlein purpura, and Reiter's disease. Moreover inflammatory diseases include connective tissue disorders such as systemic lupus erythematosus, polymyositis/dermatomyositis, systemic sclerosis, mixed connetive tissue disease,
10   polymyalgia rheumatica, and other types of vasculitis, crystal deposition diseases (including gout), pyrophosphate arthropathy, acute calcific periarthritis. Moreover inflammatory diseases include infective arthritis, juvenile arthritis (Still's disease), psoriasis, osteoarthritis, osteoarthritis secondary to hypermobilty, congenital dysplasias, slipped femoral epiphysis, Perthes' disease, intra-articular fractures, meniscectomy,
15   obesity, recurrent dislocation, repetitive actions, crystal depositions and diseases and meatobilic abnormalities of cartilage including pyrophosphate arthropathy, ochronosis, heamochromatosis, avascular necrosis including Sickle Cell disease, therapy with corticoids or other drugs, Caisson disease, septic or infectios arthitis (including tuberculous arthritis, meningococcal arthristis, gonococcal arthritis, salmonella arthritis),
20   Lymes disease, infective endocarditis (including endocarditis induced by Stretococcus viridans, Enterococcus Faecalis, Staphylococcus aureus, Staphylocossus epidermidis, Histoplasma, Brucella, Candida and Aspergellus species and Coxiella Burnetii), viral arthritis (including infection with rubella, mumps, hepatitis B, HIV or Parvovirus), or recurrent heamarthrosis. Moreover inflammatory diseases include connective tissue
25   diseases such as systemic lupus erythematosus, polymyositis/dermatomyositis, systemic sclerosis, mixed connetive tissue disease, sarcoidosis and primary Sjogrens syndrome including keratoconjunctivitis sicca. Moreover inflammatory diseases include vasculitis such as infective vasculitis due to infections with bacterial species including spirochaetal diseses as Lyme disease, syphilis, rickettsial and mycobacterial infections, fungal, viral or
30   protozoal infections. Moreover inflammatory diseases include non-infective vascultitis including Takayasu's arteritis, Giant Cell Arteritis (Temporal arteritis and polymyalgia rheumatica), Buerger's disease, polyarteritis nodosa, microscopic polyarteritis, Wegener's granulomatose, Churg-Strauss syndrome, Sarcoidosis, vasculitis secondary to connective tissue diseases including Systemic Lupus Erythematosus, Polymyositis/Dermatomyositis,
35   Systemic Sclerosis, Mixed Connetive Tissue Disease, sarcoidosis and Primary Sjogrens

syndrome. Moreover inflammatory diseases include vasculitis secondary to rheumatoid arthritis.

Moreover inflammatory diseases include non-infective vasculitis secondary to
5 hypersensibility and leucocytoclastic vascultis including Serum Sickness, Henoch-Schonlein purpura, Drug induced vasculitis, essential mixed cryoglobulinaemia, hypocomplentaemia, Vascultis associated with other kinds of malignancy, inflammatory bowel disease and primary biliary cirrhosis, Goodpsture syndrome.

10 Moreover inflammatory diseases include all kinds of arthtitis in children such as Juvenile Chronic arthritis including Still's disease, juvenile rheumatoid arthritis, juvenile ankylosing spondylitis.

Moreover inflammatory diseases include all kinds of depostion diseases as Gout,
15 pyrophosphate arthopathy and acute calcific periarthritis.

Moreover inflammatory diseases include all kind of inflammatory conditions causing backpain including infections, septic discitis, tuberculosis, malignancies (such as matastases, myeloma and others), spinal tumours, ancylosing spondylitis, acute disc
20 prolapse, chronic disc diseaase/osteoarthritis, osteoporosis, and osteomalacia. It also includes Pagets disease, hyperparathyroidism, renal osteodystrophy, spondylolisthesis, spinal senosis congenital abnormalities and fibromyalgia.

Moreover inflammatory diseases include all kinds of soft-tissue rheumatism including
25 bursitis, tenosynovitis or peritendonitis, enthesitis, nerve compression, periarthritis or capsulitis, muscle tension and muscle dysfunction.

Moreover inflammatory diseases include inflammatory diseases of the gastrointestinal system (including stomatitis of all kinds, pemfigus, bulloid pemphigoid and benig mucous
30 membrane pemphigoid), salivary gland diseases (such as sarcoidosis, salivary duct obstruction and Sjogrens syndrome), inflammaton of the oesophagus (e.g. due to gastro-oesophagel reflux or infections with candida species, herpes simplex and cytomegalus virus), inflammatory dieseases of the stomach (including acute and chronic gastritis, helicobacteer pylori infection and Mentriers disease), inflammation of the the small
35 intestine (including coeliac disease, gluten sensitive enteropathy, dermatitis herpitiformis,

tropical sprue, Whipple's diease, rdiation enteritis, systemic amyloidosis, connective tissue disorders including systemic lupus erythematosus, polymyositis/dermatomyositis, systemic sclerosis, mixed connetive tissue disease and sarcoidosis), eosinophilic gastroenteritis, intestinal lympangiectasia, inflammatory bowel disease (including Chrons

5 disease and ulcertive colitis), diverticular disease of the colon, and irritable bowel syndrome

Moreover, inflammatory diseases include upper and lower airway diseases such as chronic obstructive pulmonary diseases (COPD), allergic and non-allergic asthma, allergic

10 rhinitis, allergic and non-allergic conjunctivitis. Moreover, inflammatory diesases also include allergic and non-allergic dermatitis.

A preferred embodiment of the invention relates to treatment of inflammatory conditions or diseases under non-ischemic conditions, i.e. conditions wherein there is substantially

15 normal blood supply to the organ or organs in question.

One preferred target organ is airways and lungs known to be site for inflammation in acute respiratory diseases and in chronic and subchronic airway and lung diseases. Another preferred target organ is the kidney, including tubules and glomeruli and the urinary tract

20 system comprising ureteres, bladder, and urethra. However, also other cell types such as the prostate may be involved in an inflammatory condition, which thereby is the subject for treatment by the method according to the invention.

The cells to be treated may be one or more cell types selected from macrophages, the

25 reticulo endothelial system monocytes, neutrophil granulocytes, eosinophil granulocytes, basophil granulocytes, T-cells, B-cells, mast cells, and dendritic cells.

In its broadest concept the invention relates to any condition wherein the normal function of the organs or tissues is altered including conditions associated with ischemia, acute

30 and/or chronic inflammation, allergy, rheumatic diseases, infection including viral, fungal, bacterial infections, prions and other microbes and infectious agents known in the art. The injury may include acute and chronic injury. Chronic injury includes situations of repetitive injuries alternating with periods of complete or partial recovery of the organ(s) or tissue(s) function. The invention also relates to injury, which is associated with implantation of one

35 or more organs or other devices for transplantation. The organ can be from the individual

him or herself, the animal itself or from other individuals or animals. This includes: organ transplants, bone transplants, soft tissue implants (silicone implants), metal and plastic implants, or other medical implantable devices. Individual represents humans as well as other mammals.

In a further embodiment, the condition to be treated may be caused by a cancer or a by premalignant disorder having an impact on the organ, e.g. on the respiratory system including lung, bronchiole, upper airways, and/or on the heart and/or on the kidney and/or on the gastrointestinal system, including acute leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia, Hodgkin's disease, lymphosarcoma, myeloma, metastasizing carcinoma of any origin.

Furthermore, the condition to be treated may be caused by any disease selected from diabetes mellitus, conditions with increased fasting levels of LDL-Cholesterol, conditions with combined increased fasting levels of LDL-Cholesterol and triglycerid, conditions with increased fasting levels of triglycerid, conditions with increased fasting levels of HDL-Cholesterol, retroperitoneal fibrosis, lupus erythematosus, polyarteritis nodosa, sclerodermia, polymyositis, dermatomyositis, rheumatoid arthritis, anaphylaxis, serum sickness, hemolytic anaemia, and allergic agranulocytosis.

Many infections may have an influence on the tissue and disturb the normal function resulting in decreased performance which may be improved by administration of an effective dose of α-MSH and/or an α-MSH equivalent and EPO and/or an EPO equivalent. Such infections include infections by protozoa, virus, bacteria and fungus and include conditions such as AIDS, bacterial septicemia, systemic fungal infections, Rickettsial diseases, toxic shock syndrome, infectious mononucleosis, chlamydia thrachomatis, chlamydia psittaci, cytomegalovirus infection, campylobacter, salmonella, influenza, poliomyelitis, toxoplasmosis, Lassa Fever, Yellow Fever, billharziose, colibacteria, enterococcus, preteus, klebsiella, pseudomonas, staphylococcus aureus, staphylococcus epidermidis, candida albicans, tuberculosis, mumps, infectious mononucleosis, hepatitis and Coxackie virus

In a still further aspect, the condition to be treated may be associated with a chemical trauma involving one or more toxic substances and/or drugs. Such drugs include tricyclic

antidepressants, lithium salts, prenylamine, phenothizine derivatives, chemopreventive drugs including adriamycin. Also physical traumas including electromagnetic radiation may cause damages which can be alleviated by administration of an effective dose of an α-MSH and/or of an α-MSH equivalent and/or administration of an EPO and/or an EPO

5 equivalent according to the present invention.

The condition, which may be treated according to the present invention may further include connective tissue disease such as scleroderma, systemic lupus erythematosus or by neuromyopathic disorders such as progressive muscular dystrophy of Duchenne´s

10 type, Friedreich´s ataxia, and myotonic dystrophy. The condition may e.g. be related to the tissue of the intestine of the mammal.

The medicament in question may be administered therapeutically for treating an existing condition or prophylactically for preventing a progress of the condition, or of any symptom

15 of the condition. It may be administered prophylactically for preventing the establishment of the condition or of any symptom of the condition. The α-MSH and/or α-MSH equivalent and/or rh-EPO and/or a rh-EPO equivalent may be administered as a single dosage, as continued administration including a regimen where specific dosages are prescribed for a shorter or longer duration, or as a sequential administration similarly with many treatment

20 schedules for cancer therapy.

The origin of the condition may include anatomic abnormality of the tissue or organ, inflammatory diseases, and/or conditions caused by a chemical trauma including drugs such as adriamycin and other chemotherapeutics; electromagnetic radiation; renal and/or

25 ureteric calculi, especially when the calculi occur frequently.

The preferred target organ according to the invention is airways, lung, ureteres, kidney, bladder, urethra and the prostate gland and wherein the tissue(s) is selected from the group of lymphoid tissues, mucosa, epithelium, and endothelium. However also

30 inflammation in other organ systems or parts of organs may be succesfully treated by the methods according to the invention. Examples of such organs or organ systems which may be treated according to a method of the invention are skin, skeleton, brain and central nervous system, muscles, vessels, upper and lower respiratory tract, lungs and pleura, exocrine and endocrine glands, heart and pericardium, intestinal tract including

excretory ducts of liver and pancreas, liver, pancreas, genital organs and uterus, kidney and urinary tract including prostate.

An interesting embodiment relates to the treatment and/or prevention of infections in

5   target organs including lung and airways but also cystitis, especially interstitiel cystitis, and cystitis limited to the mucosa. The infections and inflammation may be a microbial or non-microbial. The infection or inflammation may be local or in distinct organ regions or systemic.

10 Many diseases such as rheumatic diseases, retroperitoneal fibrosis, lupus erythematosus, polyarteritis nodosa, sclerodermia, polymyositis, dermatomyositis, rheumatoid arthritis, anaphylaxis, serum sickness, hemolytic anaemia, allergic agranulocytosis, may succesfully be treated according to the present invention.

15 Another important embodiment is the conditions wherein diabetes mellitus is involved.

Yet another important embodiment is conditions associated with inflammation of skin.

Examples of conditions to be treated by the methods according to the present invention is

20 wherein the condition is lung and/or airways, kidney (recognized as renal failure, nephrotic syndrome) or complete or partial urinary tract obstruction, postoperative polyuria.

In one important embodiment the condition to be treated by the methods of the invention is associated with inflammation of lung and airways with infiltration of one or more of

25 leucocytes including neutrophils, eosinophils, lymphocytes and monocytes but also macrophages, mast cells or basophils. These conditions include chronic obstructive pulmonary diseases with or without acute infections or worsening of inflammation, allergic diseases, astma. This also includes inflammation in the airways including upper airways caused by allergy or infection or other diseases.

30

In one embodiment the condition is associated with reduced renal function indicated by one or more of the following conditions; reduced renal blood flow, reduced glomerular filtration rate, reduced urinary concentrating ability, reduced urinary concentration capacity, reduced or increased urinary electrolyte excretion (such as sodium, potassium,

bicarbonate), reduced creatinine clearance which may be treated or prevented by the methods according to the invention.

Accordingly, the condition may be associated with dysregulation of one or more renal

5   sodium transporters. Such downregulation of one or more renal sodium transporters may be of at least 50 %, such as at least 75%, compared to non-treatment. The sodium transporters may be selected from the group consisting of Na,K-ATPase, NHE-3, NaPi-2, BSC-1, TSC and ENaC's.

10   Also dysregulation of one or more renal aquaporins may characterize a condition to be treated according to the invention including downregulation of one or more renal aquaporins including aquaporins selected from aquaporins 1 to 12, preferably aquaporins 1 to 4.

15   The administration according to the use and method of the present invention may be any administration known in the art as may easily be recognised by the skilled person according to the individual situation. Accordingly, the administration may be selected from systemic administration; injection into tissue or into a to body cavity including joints; implantation into tissue or into a body cavity; topical application to the skin or to any

20   gastrointestinal surface, or to a mucosal surface including the lining of body cavities.The administration may be selected from parenteral adminstration, including intraperitonal administration, intrathecal administration systemic administration, local administration, topical administration, transmucosal administration, transdermal administration and oral administration.

25

The α-MSH equivalent according to the present invention is preferably a substance acting on an α-MSH receptor and/or on a melanocortin receptor such as subtypes 1 to 5 (MC-receptors 1-5). Such substances are disclosed in e.g. EP 972522, WO 87/04623, WO 88/00833, WO 99/57148, WO 99/21571, WO 96/41815, US 5028592, US 5,731,408, US

30   5,830,994 and the references cited therein.

In a further important aspect, the α-MSH equivalent is a polypeptide having at least 3 amino acids including the following sequence Lys-Pro-Val, such as Gly-Lys-Pro-Val, or the following sequence His-Phe-Arg, and being able to act on an α-MSH receptor.

35

The treatment or prevention as described above is in a preferred embodiment performed with EPO and/or an EPO equivalent i.e. a substance acting on the EPO receptor. In this respect, the effective dosage of the unit of EPO and/or an EPO equivalent is lower than the dosage in which EPO is generally used for its known indications. The necessary

5   dosage of EPO will generally be a completely non-toxic dosage for the individual.

By use of the combination of an α-MSH or α-MSH equivalent with rh-EPO and/or an EPO equivalent, a synergistic effect may be obtained. The synergism may during a treatment period be at least 5% or even higher such as at least 10%, preferebly at least 15% as

10 measured according to the selected test system in an organ. It is believed that a synergistic effect of at least 20%, such as at least 25%, may be demonstrated by a treatment according to the invention. The test system may be any of the experimental protocols described herein.

15 Pharmaceutical formulations and compositions:

In the following examples of suitable compositions containing α-MSH and/or an α-MSH equivalent and EPO and/or EPO equivalent are given. Depending on the use of the α-MSH and/or an α-MSH equivalent and EPO and/or EPO equivalent, a composition may

20 be a pharmaceutical or a cosmetic composition. In the following the term "pharmaceutical composition" is also intended to embrace cosmetic compositions as well as compositions belonging to the so-called grey area between pharmaceuticals and cosmetics, namely cosmeceuticals.

25 For the administration to an individual (an animal or a human) the substance(s) are preferably formulated into a pharmaceutical composition containing the substance(s) and, optionally, one or more pharmaceutically acceptable excipients.

The compositions may be in form of, e.g., solid, semi-solid or fluid compositions such as,

30 e.g., but not limited to

bioabsorbable patches, drenches, dressings, hydrogel dressings, hydrocolloid dressings, films, foams, sheets, bandages, plasters, delivery devices, implants,

powders, granules, granulates, capsules, agarose or chitosan beads, tablets, pills, pellets, microcapsules, microspheres, nanoparticles, sprays, aerosols, inhalation devices,

gels, hydrogels, pastes, ointments, creams, soaps, suppositories, vagitories, tooth
5   paste,solutions, dispersions, suspensions, emulsions, mixtures, lotions, mouthwash, shampoos, enemas,

kits containing e.g. two separate containers, wherein the first one of the containers contains the α-MSH and/or α-MSH equivalent and/or EPO and/or EPO equivalent and/or
10   pharmaceutically acceptable excipients and the second container containing a suitable medium intended to be added to the first container before use in order to obtain a ready-to-use composition;and in other suitable forms such as, e.g., implants or coating of implants or in a form suitable for use in connection with implantation or transplantation.

15   The compositions may be formulated according to conventional pharmaceutical practice, see, e.g., "Remington: The science and practice of pharmacy" 20th ed. Mack Publishing, Easton PA, 2000 ISBN 0-912734-04-3 and "Encyclopedia of Pharmaceutical Technology", edited by Swarbrick, J. & J. C. Boylan, Marcel Dekker, Inc., New York, 1988 ISBN 0-8247-2800-9.
20

A pharmaceutical composition comprising an active substance serves as a drug delivery system. In the present context the term "drug delivery system" denotes a pharmaceutical composition (a pharmaceutical formulation or a dosage form) which upon administration
25   presents the active substance to the body of a human or an animal. Thus, the term "drug delivery system" embraces plain pharmaceutical compositions such as, e.g., creams, ointments, liquids, powders, tablets, etc. as well as more sophisticated formulations such as sprays, plasters, bandages, dressings, devices, etc.

30   Apart from α-MSH and/or an α-MSH equivalent and/or EPO and/or EPO equivalent, a pharmaceutical composition for use according to the invention may comprise pharmaceutically or cosmetically acceptable excipients.

The choice of pharmaceutically acceptable excipients in a composition for use according
35   to the invention and the optimum concentration thereof cannot generally be predicted and

must be determined on the basis of an experimental determination thereof. Also whether a pharmaceutically acceptable excipient is suitable for use in a pharmaceutical composition is generally dependent on which kind of dosage form is chosen. However, a person skilled in the art of pharmaceutical formulation can find guidance in e.g.,

5 "Remington: The science and practice of pharmacy" 20th ed. Mack Publishing, Easton PA, 2000 ISBN 0-912734-04-3.

A pharmaceutically acceptable excipient is a substance, which is substantially harmless to the individual to which the composition will be administered. Such an excipient normally

10 fulfils the requirements given by the national drug agencies. Official pharmacopeias such as the British Pharmacopeia, the United States of America Pharmacopeia and the European Pharmacopeia set standards for well-known pharmaceutically acceptable excipients.

15 In the following is given a review on relevant pharmaceutical compositions for use according to the invention. The review is based on the particular route of administration. However, it is appreciated that in those cases where a pharmaceutically acceptable excipient may be employed in different dosage forms or compositions, the application of a particular pharmaceutically acceptable excipient is not limited to a particular dosage form

20 or of a particular function of the excipient.

Parenteral compositions:

For systemic application, the compositions according to the invention may contain

25 conventionally non-toxic pharmaceutically acceptable carriers and excipients according to the including microspheres and liposomes.

The compositions for use according to the invention include all kinds of solid, semisolid and fluid compositions. Compositions of particular relevance are e.g. solutions,

30 suspensions, emulsions, gels, implantation tablets and implants.

The pharmaceutically acceptable excipients may include solvents, buffering agents, preservatives, humectants, chelating agents, antioxidants, stabilizers, emulsifying agents, suspending agents, gel-forming agents, diluents, disintegratig agents, binding agents,

35 lubricants and wetting agents. For examples of the different agents see below.

Topical, trans-mucosal and trans-dermal compositions:

For application to the mucosa or the skin, the compositions for use according to the
5   invention may contain conventionally non-toxic pharmaceutically acceptable carriers and
excipients including microspheres and liposomes.

The compositions for use according to the invention include all kinds of solid, semi-solid
and fluid compositions. Compositions of particular relevance are e.g. pastes, ointments,
10 hydrophilic ointments, creams, gels, hydrogels, solutions, emulsions, suspensions,
lotions, liniments, resoriblets, suppositories, enema, pessaries, moulded pessaries,
vaginal capsules, vaginal tablets, shampoos, jellies, soaps, sticks, sprays, powders, films,
foams, pads, sponges (e.g. collagen sponges), pads, dressings (such as, e.g., absorbent
wound dressings), drenches, bandages, plasters and transdermal delivery systems.
15

The pharmaceutically acceptable excipients may include solvents, buffering agents,
preservatives, humectants, chelating agents, antioxidants, stabilizers, emulsifying agents,
suspending agents, gel-forming agents, ointment bases, suppositoriy bases, penetration
enhancers, perfumes, skin protective agents, diluents, disintegratig agents, binding
20 agents, lubricants and wetting agents. For examples of the different agents see below.

Oral compositions:

For application to the mucosa or the skin, the compositions for use according to the
25 invention may contain conventionally non-toxic pharmaceutically acceptable carriers and
excipients including microspheres and liposomes.

The composition for use according to the invention include all kinds of solid, semi-solid
and fluid compositions. Compositions of particular relevance are e.g. solutions,
30 suspensions, emulsions, uncoated tablets, modified-release tablets, gastro-resistant
tablets, orodispersible tablets, efferverscent tablets, chewable tablets, soft capsules, hard
capsules, modified.release capsules, gastro-resistant capsules, uncoated granules,
effervescent granules, granules for the preparation of liquids for oral use, coated granules,
gastro-resistant granules, modified-release granules, powders for oral administration and
35 powders for the preparation of liquids for oral use.

The pharmaceutically acceptable excipients may include solvents, buffering agents, preservatives, humectants, chelating agents, antioxidants, stabilizers, emulsifying agents, suspending agents, gel-forming agents, diluents, disintegratig agents, binding agents,

5   lubricants, coating agents and wetting agents. For examples of the different agents see below.

Examples of various agents:

10   Examples of solvents are but not limited to water, alcohols, vegetable or marine oils (e.g. edible oils like almond oil, castor oil, cacao butter, coconut oil, corn oil, cottonseed oil, linseed oil, olive oil, palm oil, peanut oil, poppyseed oil, rapeseed oil, sesame oil, soybean oil, sunflower oil, and teaseed oil), mineral oils, fatty oils, liquid paraffin, polyethylene glycols, propylene glycols, glycerol, liquid polyalkylsiloxanes, and mixtures thereof.

15

Examples of buffering agents are but not limited to citric acid, acetic acid, tartaric acid, lactic acid, hydrogenphosphoric acid, diethylamine etc.

Examples of preservatives for use in compositions are but not limited to parabens, such

20   as methyl, ethyl, propyl p-hydroxybenzoate, butylparaben, isobutylparaben, isopropylparaben, potassium sorbate, sorbic acid, benzoic acid, methyl benzoate, phenoxyethanol, bronopol, bronidox, MDM hydantoin, iodopropynyl butylcarbamate, EDTA, benzalconium chloride, and benzylalcohol, or mixtures of preservatives.

25   Examples of humectants are but not limited to glycerin, propylene glycol, sorbitol, lactic acid, urea, and mixtures thereof.

Examples of chelating agents are but not limited to sodium EDTA and citric acid.

30   Examples of antioxidants are but not limited to butylated hydroxy anisole (BHA), ascorbic acid and derivatives thereof, tocopherol and derivatives thereof, cysteine, and mixtures thereof.

Examples of emulsifying agents are but not limited to naturally occurring gums, e.g. gum

35   acacia or gum tragacanth; naturally occurring phosphatides, e.g. soybean lecithin;

sorbitan monooleate derivatives; wool fats; wool alcohols; sorbitan esters; monoglycerides; fatty alcohols;, fatty acid esters (e.g. triglycerides of fatty acids); and mixtures thereof.

5 Examples of suspending agents are but not limited to celluloses and cellulose derivatives such as, e.g., carboxymethyl cellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carraghenan, acacia gum, arabic gum, tragacanth, and mixtures thereof.

10 Examples of gel bases and viscosity-increasing are but not limited to liquid paraffin, polyethylene, fatty oils, colloidal silica or aluminium, zinc soaps, glycerol, propylene glycol, tragacanth, carboxyvinyl polymers, magnesium-aluminium silicates, Carbopol®, hydrophilic polymers such as, e.g. starch or cellulose derivatives such as, e.g., carboxymethylcellulose, hydroxyethylcellulose and other cellulose derivatives, water-

15 swellable hydrocolloids, carragenans, hyaluronates (e.g. hyaluronate gel optionally containing sodium chloride), and alginates including propylene glycol aginate.

Examples of ointment bases are but not limited to beeswax, paraffin, cetanol, cetyl palmitate, vegetable oils, sorbitan esters of fatty acids (Span), polyethylene glycols, and

20 condensation products between sorbitan esters of fatty acids and ethylene oxide, e.g. polyoxyethylene sorbitan monooleate (Tween).

Examples of hydrophobic ointment bases are but not limited to paraffins, vegetable oils, animal fats, synthetic glycerides, waxes, lanolin, and liquid polyalkylsiloxanes.

25

Examples of hydrophilic ointment bases are but not limited to solid macrogols (polyethylene glycols).

Examples of powder components are but not limited to alginate, collagen, lactose, powder

30 which is able to form a gel when applied to a wound (absorbs liquid/wound exudate).

Examples of diluents and disintegrating agents are but not limited to lactose, saccharose, emdex, calcium phosphates, calcium carbonate, calcium sulphate, mannitol, starches and microcrystaline cellulose.

35

Examples of binding agents are but not limited to saccharose, sorbitol, gum acacia, sodium alginate, gelatine, starches, cellulose, sodium coboxymethylcellulose, methylcellulose, hydroxypropylcellulose, polyvinylpyrrolidone and polyetyleneglycol.

5 Examples of wetting agents are but not limited to sodium laurylsulphate and polysorbate 80.

Examples of lubricants are but not limited to talcum, magnesium stearate, calcium stearate, silicium oxide, precirol and polyethylenglycol.

10

Examples of coating agents are but not limited to hydroxypropylcellulose, hydroxypropylmethylcellulose, polyvinylpropylidone, ethylcellulose and polymethylacrylates.

15 Examples of suppository bases are but not limited to oleum cacao, adeps solidus and polyethylenglycols.

The α-MSH and/or α-MSH equivalent and/or EPO and/or EPO equivalent is present in the medicament in an amount of 0.001-99%, typically 0.01-75%, more typically 0.1-20%,
20 especially 1-10% by weight of the medicament.

By EPO equivalent according to the present invention is meant any substance, which has a functional effect on an EPO receptor.

25 The EPO and/or EPO equivalent may be present in the medicament in an amount of 0.001-99%, typically 0.01-75%, more typically 0.1-20%, especially 1-10% by weight of the medicament. The EPO and/or EPO equivalent is generally used in dosages, which are completely non-toxic to a human. By use of a combination of an EPO and/or EPO equivalent together with a unit dosage of α-MSH and/or an α-MSH equivalent, an effect of
30 the combination may be obtained which is higher than the effect obtained with any of the substances administered alone.

With respect to the combination, it is possible to obtain an synergistic effect where the EPO and/or EPO equivalent and the α-MSH and/or α-MSH equivalent is administered
35 independently of each other. The time span from the release of one of the active

ingredients to the organ or tissue in question until the other active ingredient is subjected
to the tissue or organ may be several days, even 5 days or one week. However, the drugs
are preferable administered within about 48 hours, preferably within 24 hours, such as
within 12 hours. However, for practical reasons the active ingredients will normally be
5　substantially co-administered with considerations to differences in pharmacokinetic
・properties and speed-of-action of the compounds.

In a further embodiment the present invention relates to a pharmaceutical composition
comprising a unit dosage of EPO and/or EPO equivalent and a unit dosage of α-MSH
10　and/or of an α-MSH equivalent, optionally together with a suitable pharmaceutical carrier.
The carrier may be selected according to the specific use as disclosed above. In a further
aspect, the composition may be specifically adapted for any of the uses and methods
disclosed herein.

15　It is contemplated that the dose of α-MSH and/or α-MSH equivalent will be in the range of
1 ng to 100 mg pr. kg body weight, typically 1μg to 10 mg pr. kg body weight, more
typically 10μg to 1 mg pr. kg body weight, such as 50-500 μg pr. kg body weight; and that
the dose of EPO and/or EPO equivalent will be in the range of 0,001-10000 IU pr. kg body
weight, typically 0,1-5000 IU pr. kg body weight, more typically 1-1000 IU pr. kg body
20　weight, such as 50-500 IU pr. kg body weight.

A further aspect of the invention is a pharmaceutical kit comprising a unit dosage of EPO
and/or EPO equivalent and a unit dosage of α-MSH and/or of an α-MSH equivalent
optionally together with a suitable pharmaceutical carrier and optionally a description of
25　the specific use. The kit may comprise the α-MSH equivalent or α-MSH in any of the
forms described herein and the EPO and/or EPO equivalent may be in an identical for or
in any other form. Accordingly, the α-MSH may be present in a device for sustained effect
whereas the EPO may be present in the kit in a form suitable for injection. The specific kit
may accordingly be designed for the individual treatment of prophylactic use.
30

LEGEND TO FIGURES

Fig 1. Panel A shows an immunoblot from whole kidney reacted with affinity-purified anti-
aquaporin-1 (anti-AQP1) which revealed 29 kDa and 35-50 kDa AQP1 bands,
35　representing non-glycosylated and glycosylated forms of AQP1. Panel B shows

densitometric analysis. In response to 24 hours of BUO and 5 hours release densitometric analysis of all samples from nontreated, α-MSH treated rats with 24-hour BUO and sham-operated controls revealed that AQP1 expression decreased from 100±22% in sham operated controls to 7±2% in BUO rats without α-MSH treatment,* P<0.05. α-MSH

5   treatment significantly increased the level of AQP1 expression to 81±21% compared with nontreated rats with BUO, # P<0.05. Thus α-MSH treatment prevented the dramatic decrease in AQP1 expression in response to 24 hours of BUO and 5 hours of release.

Fig 2. Panel A: Glomerular filtration rate (GFR) did not differ among the three groups at

10   baseline level. α-MSH treatment completely prevented the reduction in GFR (BUO+MSH: 705 ± 85 μl/min/100g vs. SHAM: 840 ± 105 μl/min/100g) 48 hours after release of BUO. Panel B: Effective renal plasma flow (ERPF) did not differ among the three groups at baseline level. α-MSH treatment completely prevented the reduction in ERPF (BUO+α-MSH: 2598 ± 129 μl/min/100g vs. SHAM: 2633 ± 457 μl/min/100g) 48 hours after release

15   of BUO.

Fig 3. Determination of proteinuria levels in α-MSH or untreated PAN rats compared to SHAM rats at day 5 after PAN injection. A marked reduction is seen in proteinuria demonstrating a dramatic effect of α-MSH in preventing to a marked extent the severity in

20   the developement of nephrotic syndrome

EXAMPLES

Experimental animals

25   Studies were performed on adult male Munich Wistar rats or Spraque Dawley rats (Møllegard Breeding centre Ltd., Eiby, Denmark). The rats were maintained on a standard rodent diet (Altromin, Lage, Germany) with free access to water. During the entire experiment, rats were kept in individual metabolic cages, with a 12:12h artificial light/dark cycle, a temperature of 21°C, plus/minus 2°C. Rats were allowed to acclimatize to the

30   cages for 3 days prior to surgery.

Induction of acute lung inflammation
Studies were performed on male Spraque Dawley rats (Møllegard Breeding centre Ltd., Eiby, Denmark). The rats were maintained on a standard rodent diet (Altromin, Lage,

Germany) with free access to water. Inflammation of the lungs (asthma) was induced by LPS inhalation with the animals anesthetized using Midazolam and Hypnorm The LPS was administrated by connecting the rat to a semiclosed breathing system consisting of a MAXIN bottle filled with a mixture of LPS in saline connected to a standard inhalation

5   chamber. Each animals is exposed for 20 min with 5 mg LPS. Twenty-four hours after exposure to LPS rats were killed. Subsequently, lavage was performed by washing the lungs with PBS (5 ml) 6 times. The lung tissue was then centrifuged for 10 min (1000 rpm) and resuspended in 0.5 ml. From each sample 2 blood smears were made. They were air-dryed, fixed and counterstained for differential cell counting.

10

Induction of acute systemic inflammation
Barrier-bred and specific pathogen-free female Wistar rats (210-230 g) were obtained from the Department of Experimental Medicine, Panum Institute, University of Copenhagen, Denmark. The animals were housed in a temperature (22-24° C) and

15 moisture (40-70%) controlled room with a 12-hour light-dark cycle (light on from 6:00 A.M. to 6:00 P.M.). All animals were given free access to tap water and a pelleted rat diet containing approximately 140 mmol/kg of sodium, 275 mmol/kg potassium and 23 % protein (Altromin catalogue no. 1310, Altromin International, Lage, Germany). Rats were anesthetized with halothane-nitrous oxide and permanent medical grade Tygon catheters

20 were implanted into the abdominal aorta and into the inferior caval vein via a femoral artery and vein. After instrumentation, the animals were housed individually. All surgical procedures were performed during aseptic conditions. To relieve postoperative pain, rats were treated with buprenorfin, 0.2 mg/kg body weight i.p. Two weeks later the rats were anesthetized with halothane-nitrous oxide and a osmotic minipump (Alzet 1003D)filled

25 with a LPS solution was implanted into the abdomen. The mean infusion rate of LPS was 200 µg/kg/hour. All rats except the control rats received LPS infusion. N=5 in all groups.

Induction of bilateral ureteral obstruction
Adult Munich-Wistar rats were anesthetized with halothane and placed on a heating board

30 under an operating microscope, Through a midline abdominal incision both ureters were exposed and occluded by placing a 5-mm piece of bisected polyethylene tubing (PE-50) around the midportion of each ureter. The ureter was then occluded by tightening the tubing with a 5-0 silk ligature. 24 hours later, the obstructed ureters were decompressed by removal of the ligature and the PE-50 tubing. Done in this manner, both ureters could

be completely occluded for 24 h and without evidence of functional impairment of ureteral function and rats followed up to several days after release of obstruction.

Induction of unilateral ureteral obstruction

5   Adult Munich-Wistar rats were anaesthetized with intraperitoneal sodium pentobarbital (50 mg/kg) and placed on a heating board under an operating microscope. Through a midline abdominal incision the left ureter was exposed and a 5 mm piece of bisected polyethylene tubing (PE-50) was placed around the midportion of the ureter. The ureter was then occluded by tightening the tubing with a 5-0 Silk ligature. Twenty-four hours later, the

10   obstructed ureters were decompressed by removal of the ligature and the PE tubing. Using this technique the ureter could be completely occluded for 24 hours without evidence of subsequent functional impairment of ureteral function.

Induction of purimycin and adriamycin-induced nephrotic syndrome in rats

15   Nephrotic syndrome was induced by a single i.p. or s.c. injection of adriamycin or purimycin (at various doses) and the rats were followed for 7 days - 21 days. Development of severe nephrotic syndrome was established by monitoring the total body weight, urinary protein excretion (proteinuria) and at time of ending the experiment the volume of fluid in the abdominal cavity was determined (rats develop severe water

20   retention and ascites). The effect of α-MSH or epoetin or α-MSH combined with epoetin was determined by treatment with the compounds for the initial 3 days after adrimycin or purimycin administration.

Experimental protocols

25   The following protocols were performed:
Protocol I-1: This protocol included 1) Rats with LPS induced lung inflammation for 24 hours (n=2) and 2) control rats (n=2).
Protocol I-II: This protocol included 1) Rats with LPS induced lung inflammation for 24 hours which were divided into the following treatment groups: 1) rats were treated with

30   saline at onset of LPS induction and after 12 hours (n=6), 2) rats were treated with α-MSH (Phoenix Pharmaceutical Inc, Mountain View, CA, 50μg, i.v.) at onset of LPS induction and after 12 hours (n=6), 3) rats were treated with Epoitin (100 U/kg/day i.p.) at LPS induction and after 12 hours and 4) rats treated with a combination of epoitin (100 U/kg/day i.p.) and α-MSH (50μg, i.v.) at the onset of LPS and 12 hours later.

35

Protocol II: Systemic LPS-infusion:

II-1. Controls. Untreated rats without LPS infusion.

II-2 Vehicle: Rats receiving LPS infusion treated with 0.5 ml 0.9 % NaCl twice daily.

II-3 rh-EPO: Rats receiving LPS infusion treated with 200 I.U. epoitin alpha (EPO)/kg body

5 weight in 0.5 ml 0.9% NaCl twice daily.

II-4 α-MSH: Rats receiving LPS infusion treated with 200 μg α-melanocyte stimulating

hormone (α-MSH)/kg body weight in 0.5 ml 0.9% NaCl twice daily.

II-4 α-MSH+rh-EPO: Rats receiving LPS infusion treated with 200 μg α-MSH/kg body

weightand 200 I.U.EPO/kg body weight in 0.5 ml 0.9% NaCl twice daily.

10 For protocols II-3, II-4 and II-5 the compounds (α-MSH and/or rh-EPO) were given i.v..

The first injections were given 12 hours after the implantation of the osmotic minipump.

Protocol III. Obstruction of the urinary tract

Protocol III-1: This protocol included 1) Rats with BUO for 24 hours (n=20) and 2) sham-

15 operated rats (n=10). The BUO animals were divided into two groups: α-MSH nontreated

(n=10) and α-MSH treated (n=10). α-MSH (Phoenix Pharmaceutical Inc, Mountain View,

CA, 50μg, i.v.) was given at the onset of BUO and 12 hours later.

Protocol III-2: This protocol included 1) Rats with BUO for 24 hours and followed by

release for 5 hours (n=10) and 2) Sham operated rats (n=5). The rats with BUO-R were

20 divided into two groups: α-MSH nontreated (n=5) and α-MSH treated (n=5). α-MSH

(50±g, i.v.) was given at the onset of BUO, 12 hours later and at the onset of release.

Protocol III-3: This protocol included 1) Rats with BUO for 24 hours and followed by

release for 48 hours. The rats were divided into two groups: α-MSH nontreated (n=10)

and α-MSH treated (n=13). α-MSH (50μg) was given with micro-osmotic pump via jugular

25 vein at the onset of BUO. 2) Sham operated rats (n=8) treated with vehicle with micro-

osmotic pump.

Protocol III-4: This protocol included rats, which had a detailed examination of renal

function before onset and after release of BUO. 1) Rats with BUO for 24 hours followed by

release for 48 hours (n=10) and 2) sham-operated rats (n=5). The BUO animals were

30 divided into two groups: α-MSH nontreated (n=5) and α-MSH treated (n=5). α-MSH

(Phoenix Pharmaceutical Inc, Mountain View, CA, 50μg, i.v.) was given at the onset of

BUO and 12 hours later.

Protocol III-5: This protocol included 1) Rats with UUO for 24 hours (n=11) and 2) sham-

operated rats (n=5). The UUO animals were divided into two groups: α-MSH nontreated

(n=5) and α-MSH treated (n=6). α-MSH (Phoenix Pharmaceutical Inc, Mountain View, CA, 50µg, i.v.) was given at the onset of UUO and 12 hours later.

Protocol III-6: This protocol included 1) Rats with BUO for 24 hours and 2) sham-operated rats. The BUO animals were divided into 4 groups: Non-treated rats (n=4), α-

5   MSH treated (n=4)(α-MSH (Phoenix Pharmaceutical Inc, Mountain View, CA, 50µg, i.v.) was given at the onset of BUO and 12 hours later), Epoitin treated rats (n=4)(100 U/kg/day i.p.) and rats treated with a combination of epoitin (100 U/kg/day i.p.) and α-MSH (50µg, i.v.) was given at the onset of BUO and 12 hours later.

10   Protocol IV: Experimental nephritic syndrome.

Protocol IV-1. Rats were treated with adriamycin (7.5 mg/kg i.p.; n=12) and followed for 21 days, respectively. Shams received saline i.p. (n=6). Half of the adriamycin treated animals received α-MSH in osmotic minipumps (50 ug/day) during the entire experiments. The other half received vehicle (saline) in osmotic minipumps. In another series of

15   experiments α-MSH treatment was administered (at the time of adriamycin injection and 6, 24 and 48 hours after adriamycin injection).

Protocol IV-2. Rats were treated with purimycin (100 mg/kg i.p., n=6) and followed for 11days, respectively. Half recieved α-MSH treatment (at the time of PAN injection and 6, 24 and 48 hours after PAN injection).

20   Protocol IV-3. Rats were treated with adriamycin (7.5 mg/kg i.p.; n=8) and followed for 21 days, respectively. Shams received saline i.p. (n=6). Half of the adriamycin treated animals received epoetin (100 units/kg/day) during the entire experiments. The other half received vehicle (saline).

Protocol IV-4. Rats were treated with purimycin aminoglycoside (100 mg/kg i.p., n=6) and

25   followed for 11 days, respectively.Half recieved epoetin (100 units/kg/day).

Protocol IV-5. Rats were treated with purimycin (100 mg/kg i.p., n=6) and followed for 11days, respectively.Half recieved α-MSH treatment (at the time of PAN injection and 6, 24 and 48 hours after PAN injection) and in addition they received epoetin (100 units/kg/day).

30

Operative procedures:

Catheterisation of the Jugular Vein

The jugular vein was exposed at least 1 cm. The tip of the catheter was inserted into the

35   vein and pushed   forwards towards the heart (about 2.5cm) filled with a heparin-saline

solution ("heparin lock"). The catheter was then tied into the blood vessel with the ligature. With a small incision made in the dorsal nape of the neck the catheter was passed subcutaneously from the site of the entry of the catheter of the jugular vein to the dorsal incision down the side of the neck to emerge anterior. And a micro-osmotic pump was
5   connected with the end of catheter.

Permanent bladder catherization

Catheters were permanently placed in the bladder for urine collection. One week before the experiment, the animals were anesthetized with halothane/N_2O. Using aseptic surgical
10 techniques, sterile Tygon™ catheters (Norton Performance Plastics, Arkon, OH) were advanced into the abdominal aorta and the inferior vena cava via the femoral vessels. A sterile chronic suprapubic catheter was implanted into the bladder. After instrumentation, the rats were infused with saline subcutaneously (5 ml) and given a long-acting analgesic, Buprenorphinum (Temgesic™ ; Reckitt & Colman, Hull, United Kingdom), subcutaneously
15 and housed individually. After a recovery period of 5 to 6 d, the rats were acclimatized to restriction by daily training sessions in restraining cages. The duration of each daily session was gradually increased from 1 to 3 h a day.

Primary Antibodies

20 For semiquantitative immunoblotting, previously characterized mouse monoclonal and affinity purified rabbit polyclonal antibodies were used:

1) AQP2 (LL127 1:6000): An affinity purified polyclonal antibody to AQP2

2) AQP1 (LL266 1:3000): An affinity purified polyclonal antibody to AQP1

3) AQP3 (LL178 1:400): An affinity purified polyclonal antibody to AQP3.

25 4) AQP4 (LL182AP): An affinity purified polyclonal antibody to AQP4 has previously been characterized.

4) NHE-3 (LL546AP): An affinity purified polyclonal antibody to NHE-3 has previously been characterized.

5) NaPi-2 (LL696AP): An affinity purified polyclonal antibody to type II Na-Pi cotransporter
30 (NaPi-2) which was raised against the final 24 amino acids of COOH-terminal sequence has previously been characterized.

6) Na,K-ATPase: A monoclonal antibody against the α-1 subunit of Na,K-ATPase has previously been characterized.

7) BSC-1 (LL320AP): An affinity purified polyclonal antibody to the apical Na-K-2Cl
35 cotransporter of the thick ascending limb has previously been characterized.

8) TSC (LL573AP): An affinity purified polyclonal antibody to the apical thiazide-sensitive Na-Cl cotransporter of the distal convoluted tubule has previously been characterized.

Clearance studies

5 Weight, water intake, food intake and urine output were observed during the rats were maintained in the metabolic cages. Urine was collected over 24-h periods throughout the study. Urine volume, osmolality, creatinine, sodium and potassium concentration were measured. Plasma was collected from abdominal aorta at the time of sacrifice for measurement of sodium and potassium concentration, creatinine, and osmolality.

10 In protocols 4 and 9 detailed examinations of renal function was performed: The experiments were carried out between 8 a.m. and 1 p.m. The rats were transferred to a restraining cage and connected to infusion pumps via the vein catheter and to a BP transducer via the arterial catheter. Urine was collected in three periods of 20 min preceded by an equilibration period of 105 min. Throughout the experiment, a half isotone

15 saline (77 mM NaCl) was infused at a rate of 70 µl/min to maintain a minimum urine flow necessary for accuracy of the bladder emptying. ^{14}C-tetraethylammonium bromide (0.83 µCi/ml ; New England Nuclear, Boston, MA), together with ^{3}H-inulin (2.5 µCi/ml ; Amersham, Rainham, United Kindgom) and LiCl (13 mmol/L), were infused together with the saline as markers of effective renal plasma flow (ERPF), GFR, and tubular fluid

20 delivery from proximal tubules (V_{prox}), respectively. A bolus of markers four times the continuous infusion velocity was given in the first 15 min. Blood samples (200 µl) were drawn from the arterial catheter after 105 and 165 min. Blood substitution with donor blood was given after each blood sample. Mean arterial BP was recorded continuously using a Uniflow™ transducer (Baxter, Irvine, CA) connected to a preamplifier and PC registration.

25 Clearance experiments were carried out in BUO and SHAM rats 7 days prior to obstruction and 48 hours after release of BUO.

Analysis

Urine volume was determined by gravimetric means. Li$^+$ concentration was determined in

30 plasma and urine by flame emission photometry and atomic absorption spectrophotometry, respectively. ^{14}C-tetraethylammonium (TEA) and ^{3}H-inulin in plasma and urine were determined by dual label liquid scintillation counting (Wallac™ model 1409; Helsinki, Finland). Sample (15 µl) and 285 µl of water were mixed with 2.5 ml of scintillation liquid (Ultima Gold™ ; Packard Instruments, Meriden, CT). Correction of dpm

35 was performed by automatic efficiency control.

Calculations

Renal clearances (*C*) were calculated by the standard formula:

$C = U \times V/P$

5 where *U* is urine concentration, *V* is urine flow rate, and *P* is plasma concentration.

In previous studies, the renal extraction fraction of TEA has been shown to approximate 90%, and the validity of TEA as an estimate of ERPF has previously been documented. By use of C_{TEA}, C_{IN}, and C_{LI}, the following parameters were calculated:

ERPF = Effective renal plasma flow (CTEA)

10 GFR = Glomerular filtration rate (Cin)

Membrane fractionation for immunoblotting

Inner medulla and whole kidneys were homogenized (0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, pH 7.2, containing 8.5 µM leupeptin. 1 mM phenylmethyl sulfonylfluoride)

15 using an ultra-turrax T8 homogenizer (IKA Labortechnik, Germany), at 5 strokes for 20 seconds (inner medulla) or at 5.5 strokes for 30 seconds (whole kidney) and the homogenate was centrifuged in an Eppendorf centrifuge at 4000 g for 15 minutes at 4°C to remove whole cells, nuclei and mitochondria. Gel samples (Laemmli sample buffer containing 2% SDS) were made of this pellet.

20

Electrophoresis and immunoblotting

Samples of membrane fractions from inner medulla and whole kidney were run on 12% or 8-16% gradient polyacrylamide minigels (Bio-Rad Mini Protean II) for AQP1, AQP2 and AQP3 (or other renal transporters). For each gel, identical gel was run in parallel and

25 subjected to Coomassie staining to assure identical loading. Then gels were subjected to immunoblotting. After transfer by electroelution to nitrocellulose membranes, blots were blocked with 5% milk in PBS-T (80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, 0.1% Tween 20, pH 7.5) for 1 h and incubated overnight at 4°C with affinity-purified primary antibodies (see above). The labeling was visualized with horseradish peroxidase (HRP)-

30 conjugated secondary antibodies (P448, DAKO, Glostrup Denmark, diluted as 1:3000) using an enhanced chemiluminescence system (ECL, Amersham International, UK).

Quantitation of total kidney levels of AQPs and other renal transporters.

ECL films with bands within the linear range were scanned using an AGFA scanner

35 (ARCUS II) and Corel Photopaint Software to control the scanner. For AQP1 and AQP2,

both the 29-kDa and the 35- to 50-kDa bands (corresponding to nonglycosylated and the glycosylated species) were scanned. For AQP3, both the 27-kDa and the 33- to 40-kDa bands (corresponding to nonglycosylated and the glycosylated species) were scanned. The labeling density was determined of blots where samples of kidneys from α-MSH

5 treated and nontreated groups were run together with samples from control kidneys. The labeling density was corrected by densitometry of coomassie stained gels. (i.e. to control for minor difference in loading)

Quantitation of urinary protein excretion

10 To determine the levels of proteinuria in experimental nephrotic syndrome urine was examined daily using Albym (Boehringer-Mannheim).

Statistical analysesValues were presented in the text as means ± standard errors. Comparisons between groups were made by unpaired t-test. *P* values < 0.05 were

15 considered significant.

Results

LPS inhalation for 20 min is associated with a marked inflammatory response in the lung

20 *tissue*
LPS induced a dramatic influx of inflammatory cells in the lung tissue. Differential counting revealed that lung tissue exposed for LPS had large numbers of neutrophil leucocytes (68.5±8%), eosinofiles (8.6±4 %), lymphocytes (15.3 ±6%), and monocytes (8±2%) (Table 1).

25

α-MSH prevents influx of inflammatory cells in lung tissue exposed with LPS
Treatment with α-MSH reduced the influx of inflammatory cells in the lung tissue exposed with LPS. Differential counting revealed that the number of eosinofils were reduced (1.8±1.3% vs. 3.6±2.7%), and neutrophil granulocytes were markedly reduced

30 (10.6±10.6% vs. 34.4±26.1%) (Table 1).

Epoitin prevents influx of inflammatory cells in lung tissue exposed with LPS
Treatment with epoitin reduced the influx of inflammatory cells in the lung tissue exposed with LPS. Differential counting revealed that the number of eosinofils were reduced

(0.7±0.6% vs. 3.6±2.7%), and neutrophil granulocytes were markedly reduced (10.9±12.7% vs. 34.4±26.1%) (Table 1).

Combined treatment with α-MSH and epoitin almost completely prevented the influx of
5 *inflammatory cells in lung tissue exposed with LPS*
Combined treatment with α-MSH and epoitin dramatically reduced the influx of inflammatory cells in the lung tissue exposed with LPS. Differential counting revealed that the number of eosinofiles were reduced (0.6±0.51% vs. 3.6±2.7%), and neutrophile granulocytes were markedly reduced (3.8±1.9% vs. 34.4±26.1%) (Table 1). In addition the
10 influx of monocytes was also significantly prevented with this treatment (6.0±4.1% vs. 13.9±8.4%) (Table 1).

LPS administration in osmotic minipumps for 3 days is associated with a severe
inflammatory response and severe renal failure
15 The rats were followed for three days after the implantation of the osmotic minipump with LPS. Glomerular filtration rate as evaluated through creatinine clearance was significantly reduced with 68% in the LPS-Vehicle group compared to the untreated control rats. Thus the animals had severe renal failure. The mortality was 20%.

20 *Treatment with α-MSH or with epoitin or with combined α-MSH and epoitin   prevented*
the inflammatory-induced renal failure seen in response to LPS administration
During 3 days of LPS-administration the mortality in the vehicle treated group were 20% (1 out of 5). All animals survived in the other groups, i.e. rh-EPO treatment, α-MSH treatment; combined treatment with low dose rh-EPO and α-MSH, and in the sham-
25 operated controls. The parameters of renal function and other parameters are described in Table 2. Glomerular filtration rate as evaluated through creatinin clearance was significantly reduced with 68% in the LPS-Vehicle group compared to the untreated control rats. Both rh-EPO and α-MSH treatment increased GFR in the LPS treated rats by 51% and 57%, respectively. However GFR was still severely affected compared to the
30 untreated control rats. The combined treatment with rh-EPO and α-MSH significantly increased GFR by 111% compared to the LPS-Vehicle group suggesting a synergestic effect of the combined treatment with rh-EPO and α-MSH.

BUO for 24 hours, 5 and 48 hours after release of BUO are associated with reduced AQP1, AQP2 and AQP3

As previously studied, immunoblotting revealed that 24 h of BUO and 48 hours after release of BUO were associated with a significant downregulation of AQP-2 expression

5   compared with sham-operated controls (13±4%). Semiquantitative immunoblotting from the inner medulla of rats with 24h BUO and BUO-R for, 5 and 48 hours revealed that AQP3 expression was persistently downregulated. Both AQP3 bands (the 27-kDa and 33- to 40-kDa bands) were decreased proportionately. Densitometric analysis revealed a significant decrease in AQP3 expression in rats with 24 h BUO to 19±4% of sham levels

10 (100±7%, $p < 0.05$). Furthermore, AQP3 protein levels were marked decreased at 5 hours after release to 15±5% of sham levels (100±9%, $p < 0.05$) and at 48 hours after release to 10±5% of sham level (100±1%, $p < 0.05$).

Semiquantitative immunoblotting using membrane fractions prepared from the whole

15 kidney of rats with 24h BUO and BUO-R for 5 and 48 hours revealed that AQP1 expression was persistently downregulated. Both AQP1 bands (the 29-kDa and 35- to 50-kDa bands) were decreased proportionately. Densitometric analysis revealed a significant decrease in AQP1 expression in rats with 24 h BUO to 53±7% of sham levels (100±9%, $p < 0.05$). Furthermore, AQP1 protein levels remained markedly decreased at 5 hours after

20 release of BUO to 7 ±2% of sham levels (100±22%, $p < 0.05$) and at 48 hours after release to 30±5% of sham level (100±10%, $p < 0.05$).

BUO for 24 hours, 5 and 48 hours after release of BUO are associated with downregulation of Na,K-ATPase

25 Semiquantitative immunoblotting using membrane fraction prepared from whole kidney of rats with 24 h BUO, and BUO followed by 5 h and 48 h after release of BUO showed a persistant downregulation of Na,K-ATPase to 35 - 50% of control levels.

α-MSH partially prevents AQP2 and AQP3 downregulation in response to 24 h of BUO

30 Semiquantitative immunoblotting using membrane fractions prepared from the inner medulla of rats with 24 h BUO and sham operated control rats revealed that α-MSH treatment significantly increased AQP2 expression were compared with nontreated rats (38±5% vs. 13±4%, $p < 0.05$). At the same time, immunoblotting also showed that

expression of AQP3 in rats with 24 h BUO was significantly upregulated in response to α-MSH treatment compared with nontreated rats (44±3% vs. 19±4%, p<0.05).

Plasma concentrations data showed that in α-MSH treated rats with 24 h BUO plasma
5   sodium levels had a marked increase compared with 24 h BUO rats without α-MSH treatment from 135±2mmol/L to 139±0.6 mmol/L. There was no difference between α-MSH treated rats and sham operated rats.

α-MSH prevents AQP3 and AQP1 downregulation in response to 24 h BUO followed by
10   *release for 5 hours*
Semiquantitative immunoblotting using membrane fractions prepared from the inner medulla of 24 h BUO rats followed by release for 5 hours and sham operated rats revealed that with α-MSH treatment AQP3 expression was significantly increased compared with nontreated rats with 24 h BUO followed by release for 5 hours form 14±5%
15   to 34±4% of sham level(100±14%).
Semiquantitative immunoblotting using membrane fractions prepared from whole kidney of rats with 24 h BUO followed by release for 5 hours and sham operated control rats revealed that with α-MSH treatment significantly increased AQP1 expression levels compared with nontreated BUO-R rats (81±21% vs. 7±2%, p<0.05) (Fig 2 A and B).
20   Furthermore, immunoblotting prepared from outer medulla and cortex also showed the same result that in α-MSH treated rats AQP1 expression was a marked increase significantly compared with nontreated rats from 35±2% to 62±9% of sham level.

α-MSH prevents AQP1 downregulation in response to 24 h BUO followed by release for
25   *48 hours*
In rats with 24 h BUO and 5 hours after release of BUO, α-MSH was given every 12 hours. In rats with 48 hours after release of BUO, α-MSH was given with micro-osmotic pump continuously. Semiquantitative immunoblotting using membrane fractions prepared from the whole kidney of 24 h BUO rats followed by release for 48 hours and sham
30   operated rats revealed that with α-MSH treatment the levels of AQP1 expression were significantly increased compared with nontreated rats with 48 hours after release of BUO form 24±5% to 58±6% of sham level (100±10%). Furthermore, immunoblotting prepared from outer medulla and cortex also showed the same result that in α-MSH treated rats

AQP1 expression was a marked increase compared with nontreated rats to 73±8% of
sham level.

α-MSH completely normalise expression of Na,K-ATPase during BUO and 5 and 48 hours
5 *after release of BUO*

Semiquantitative immunoblotting using membrane fractions prepared from whole kidney
of rats with 24 h BUO showed that Na,K-ATPase levels were completely normalized. Also,
24 h BUO followed by 5 h of release and sham operated control rats revealed that α-MSH
completely normalized Na-K-ATPase levels (102±14%). α-MSH treatment also
10 completely normalized Na-K-ATPase levels 48 hours after release of BUO (114±10%).

Treatment with α-MSH prevents the increase in plasma creatinine and restores GFR and
RPF in response to 24 hours of BUO followed by 48 hours of release

Plasma creatinine is an important marker of glomerular filtration rate. In rats with BUO for
15 24 h followed by 48 of release and sham operated control rats α-MSH treatment
significantly prevented the dramatic increase in plasma creatinine (indicating severe renal
insufficiency). Plasma creatinine levels were significantly reduced in α-MSH treated rats
(79±34 μmol/l vs.160±34 μmol/l, p<0.05,). In addition, plasma urea which is another
important marker of renal function almost normalized in rats treated with α-MSH (13±2
20 mmol/l vs. 35±9 mmol/l, p<0.05). Also GFR and ERPF were completely normalized (Fig 3
A and B).

Epoitin partially prevents AQP2 downregulation in response to 24 h of BUO

Semiquantitative immunoblotting using membrane fractions prepared from the inner
25 medulla of rats with 24 h BUO and sham operated control rats revealed that epoitin
treatment significantly increased AQP2 expression were compared with nontreated rats
(30±18% vs. 16±3%, p<0.05).

Preventive effect of α-MSH, or Epoetin treatment on adriamycin and purimycin-induced
30 *nephrotic syndrome in rats.*

Treatment with α-MSH for 3-4 days after i.p injection of adriamycin (Protocol IV-1)
significantly prevented the ascites production by 89 ± 7% indicating a marked preventive
effect of α-MSH on experimentally induced nephrotic syndrome. A similar dramatic effect
was also observed in low dose purimycin treatment (80±8% reduction, n=6) and a marked

reduction in proteinuria (Protocol IV-2). A less potent effect was seen in very servere purimycin (high dose) induced nephrotic syndrome. EPO treatment once every 24 hours also prevented the ascites formation in adriamycin-induced nephrotic syndrome and co-treatment of epoetin and α-MSH produced an even greater effect.

5

TABLES

Table 1.

A) Differential counting showed that LPS induced a marked inflammatory response in the

B) Differential counting showed marked reduction in the number of inflammatory cells with all three treatment regimens. Importantly the data show a dramatic reduction in response to combined treatment with α-MSH+Epoitin.

A	Differential counting of cells %			
Treatment	Eosinofiles	Neutrofiles	Lymfocytes	Monocytes
Controls	0	0	0	0
LPS	8.6±4	68.5±8	15.2 ±6	8±2

B	Differential counting of cells %			
Treatment	Eosinofiles	Neutrofiles	Lymfocytes	Monocytes
Vehicle	3.6±2.7	34.4±26.1	48.5±20.9	13.9±8.4
α-MSH	1.8±1.3	10.6±10.6	72.3±12.1	12.3±7.4
Epoitin	0.7±0.6	10.9±12.7	77.1±9.9	11.4±4.6
α-MSH+Epoitin	0.6±0.51	3.8±1.9	89.4±4.1	6.0±4.1

Table 2. Effects of vehicle (0.9% NaCl); rh-EPO (200 U/kg b.w.); α-MSH (200 µg/kg b.w.) and the combination of rh-EPO (200 U/kg b.w.) and α-MSH (200 µg/kg b.w.) on renal function in rats treated with continious LPS infusion (200 µg/kg/h) during three days.

	Creatinin Clearance ml/min
Control:	1.11 ± 0.08
Vehicle:	0.35 ± 0.07
rh-EPO:	0.53 ± 0.06 *
α-MSH:	0.55 ± 0.05 *
α-MSH+rh-EPO:	0.74 ± 0.06 * # ¤:

5 *: different from Vehicle treated rats; $p < 0.05$; #: different from rh-EPO treated rats; $p < 0.05$;

¤: different from α-MSH treated rats; $p < 0.05$.

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CLAIMS

1. Method for treatment or prevention of a non-ischemic condition in one or more organ(s) or tissue(s), the method comprising administering of an effective dosage of α-MSH and/or

5   of an α-MSH equivalent and/or a dosage of EPO and/or an EPO equivalent to the individual in need thereof.

2. Method according to claim 1 wherein the dosage of α-MSH and/or of an α-MSH equivalent and/or EPO and/or an EPO equivalent is administered prophylactically for

10 preventing the establishment or progress of the condition, or of any symptom of the condition.

3. Method for treatment or prevention of an inflammatory condition in one or more organ(s) or tissue(s), the method comprising administering of an effective dosage of α-

15 MSH and/or of an α-MSH equivalent and/or a dosage of EPO and/or an EPO equivalent to the individual in need thereof.

4. Method according to claim 3 wherein the dosage of α-MSH and/or of an α-MSH equivalent and/or EPO and/or an EPO equivalent is administered prophylactically for

20 preventing the establishment or progress of the condition, or of any symptom of the condition.

5. Method according to claim 1 or 3 wherein the dosage of α-MSH and/or of an α-MSH equivalent and EPO and/or an EPO equivalent is administered as a single dosage,

25 regular or continued administration, or as a sequential administration.

6. Method according to claim 1 or 3 wherein condition is caused by an infection.

7. Method according to claim 1 or 3 wherein the condition is caused by a cancer or a by

30 premalignant disorder.

8. Method according to claim 1 or 3 wherein the α-MSH equivalent is a substance acting on the α-MSH receptor and/or on the melanocortin receptor.

9. Method according to claim 1 or 3 wherein the treatment or prevention comprises administration of a dosage unit of EPO and/or an EPO equivalent.

10. Method according to claim 1 or 3 wherein a combination of α-MSH and/or α-MSH
5   equivalent with EPO and/or an EPO equivalent is adminstered.

11. Use of α-MSH and/or an equivalent of α-MSH and/or EPO and/or an EPO equivalent for the preparation of a medicament for treatment or prevention of a non-ischemic condition.
10

12. A pharmaceutical composition comprising a unit dosage of EPO and/or EPO equivalent and a unit dosage of α-MSH and/or of an α-MSH equivalent together with a suitable pharmaceutical carrier.
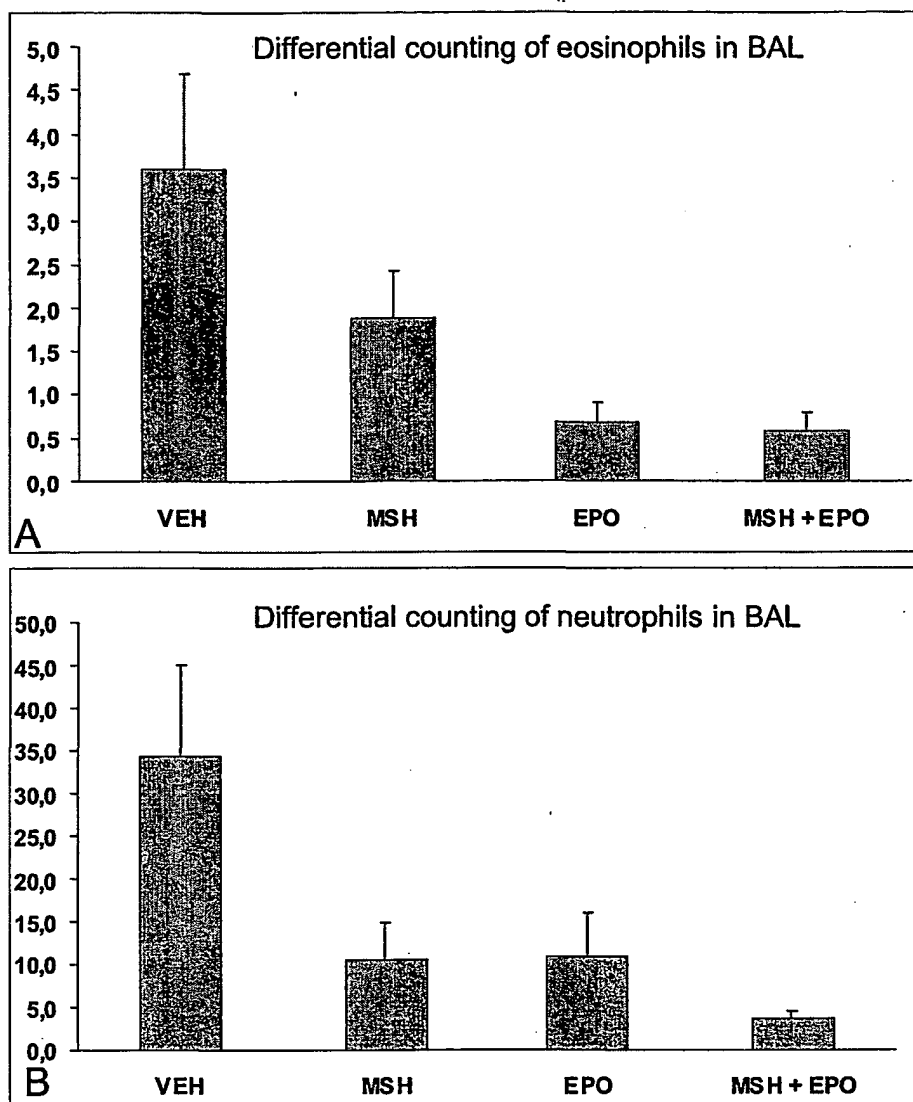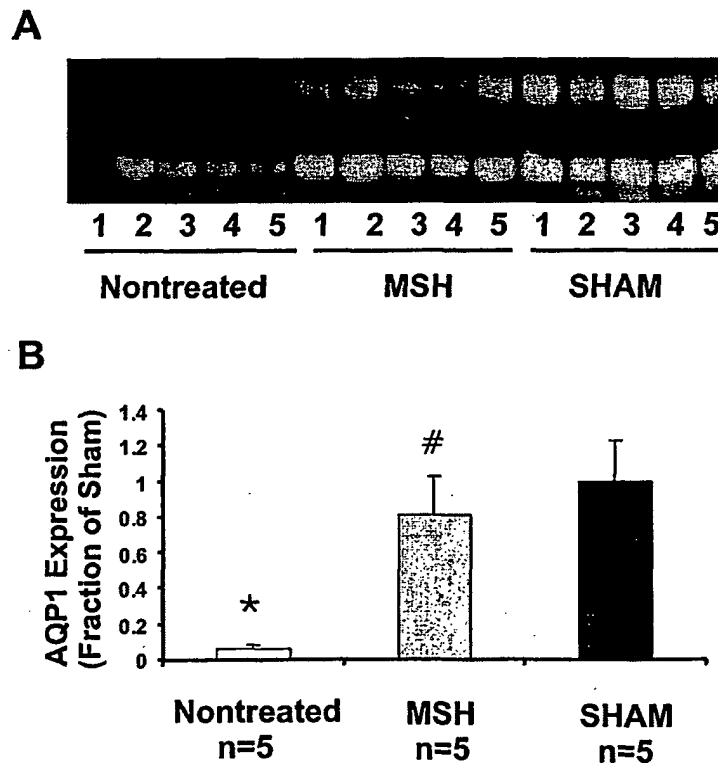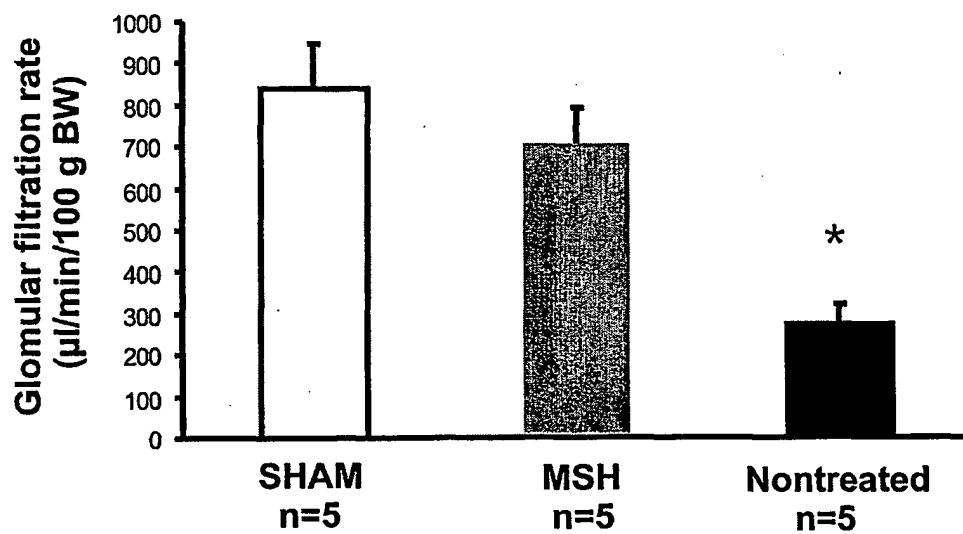
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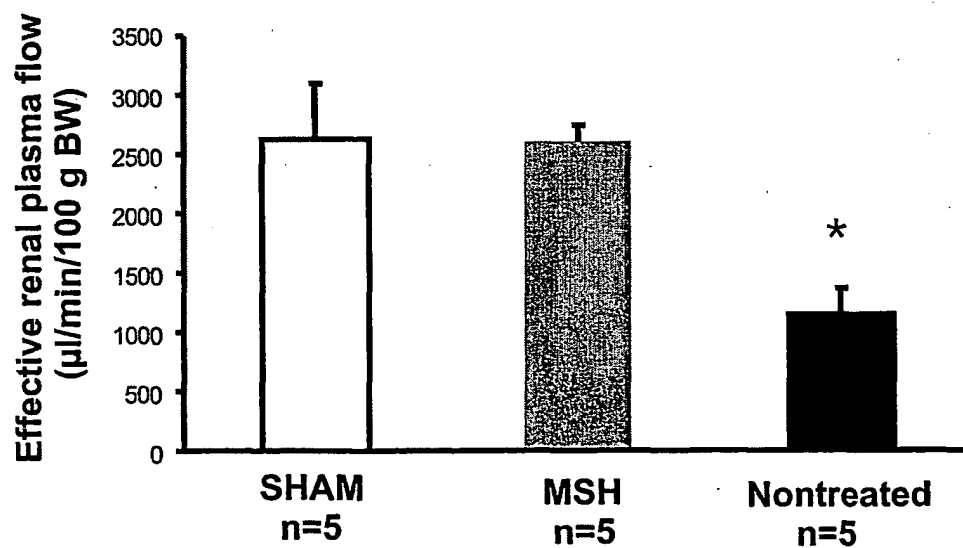
2/3

A


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 1  2  3  4  5   1  2  3  4  5   1  2  3  4  5
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Nontreated   **MSH**   **SHAM**

B

Fig. 2   BEST AVAILABLE COPY

A

B

Fig. 3   BEST AVAILABLE COPY